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Original Article

Study of the integument that covering back and stinger of the freshwater stingray *Potamotrygon rex* (Chondricthyes, Potamotrygonidae)

Estudo do tegumento que reveste o dorso e ferrão da arraia de água doce *Potamotrygon rex* (Chondricthyes, Potamotrygonidae)

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Abstract

The objective of this study was to describe the histology and histochemistry of the integument covering the back and stinger of the freshwater stingray *Potamotrygon rex*, endemic to the Middle Upper Tocantins River. The species has a dark back and yellowish circular spots that extend to the tail, which has one to two stings located in the median portion of the tail. Through histological study it was observed that the epithelia of the back and stinger are composed of non-keratinized stratified pavement epithelial tissue, and are organized in three layers: basal, intermediate and superficial. The differences between the tissues are related to the cell types that compose them. The back is organized with epithelial cells, mucus cells, granulocyte cells and chromatophores. The mucus cells are distributed in different layers along the animal's back, influencing the thickness of the tissue. The tissue that covers the stinger is composed of epithelial cells, chromatophores and specialized cells in protein synthesis. In the histochemistry, the stinger epithelial cells were stained with Bromophenol Blue, especially those of the intermediate layer, which were called specialized cells. In the back the epithelial cells were stained with Bromophenol Blue, Alcian Blue and PAS, and the mucous cells with PAS. In both tissues the presence of protein reserves was detected, areas so called because they are stained strongly with Bromophenol Blue. The results show that the stinger presents activity directed to the production of proteins, and that the back is organized to produce different components, which constitute the cuticle that covers the animal's body.

keywords: chondrichthyes, histochemistry, histology.

Resumo

O objetivo deste estudo foi descrever a histologia e histoquímica do tegumento que reveste o dorso e o ferrão da arraia de água doce *Potamotrygon rex*, endêmica do Médio Alto Tocantins. A espécie possui o dorso escuro e manchas circulares amareladas que se estendem até a cauda, que possui de um a dois ferrões localizados na porção mediana da cauda. Através do estudo histológico observou-se que os epitélios do dorso e do ferrão são compostos por tecido epitelial pavimentoso estratificado não queratinizado, e estão organizados em três camadas: basal, intermediária e superficial. As diferenças entre os tecidos estão relacionadas aos tipos de células que os compõem. O dorso é organizado com células epiteliais, células mucosas, células de granulócitos e cromatóforos. As células mucosas estão distribuídas em diferentes camadas ao longo do dorso do animal, influenciando na espessura do tecido. O tecido que reveste o ferrão é composto por células epiteliais, cromatóforos e células proteicas. Na histoquímica, as células epiteliais do ferrão foram coradas com Azul de Bromofenol, principalmente as da camada intermediária, que foram denominadas células proteicas. No dorso as células epiteliais foram coradas com Azul de Bromofenol, Azul Alcian e PAS, e as células mucosas com PAS. Em ambos os tecidos também foi detectada a presença de reservatórios de proteínas, áreas assim denominadas por estarem fortemente coradas com Azul de Bromofenol. Os resultados mostram que o ferrão apresenta atividade direcionada à produção de proteínas, e que o dorso se organiza para produzir diferentes componentes que constituem a cutícula que reveste o corpo do animal.

Palavras-chave: chondrichthyes, histoquímica, histologia.

1. Introduction

Stingrays are cartilaginous fish belonging to the Elasmobranch subclass and can inhabit from the open sea to estuarine and freshwater environments. The species has a flat body with highly developed pectoral fins and covered

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by placoid scales, which are of dermal origin, composed of enamel, dentin, vessels, and nerves (Rosa and Carvalho, 2007; Carvalho and Lovejoy, 2011; Moro et al., 2012).

Potamotrigonids are freshwater stingrays, benthic, and always live buried under the sand (Garrone-Neto and Uieda, 2012, Oliveira et al., 2017), which allows the accumulation of decomposing organic sediments on their back. The integument, as well as the secretions it produces, can be considered the animal's first line of defense against pathogens, in addition to participating in the regulation of several physiological processes such as ionic, osmotic, locomotion, and reproduction (Shephard, 1994; Zhao et al., 2008).

The epiderms in elasmobranchs can present four to eight layers of cells, which can vary depending on the region of the body or the species (Seegers and Meyer, 2009). When comparing the epidermis of sharks and stingray, many columnar secretory cells are seen as the dominant epidermal feature. In stingrays, the epidermis is more compact and partially homogeneous, and the distribution of columnar cells is irregular throughout the body (Meyer and Seegers, 2012). Besides this, few studies portray the structure and function of the elasmobranch epidermis (Meyer et al., 2007; Rakers et al., 2010; Elliott, 2011a).

Stingray accidents are frequent in the Amazon and Tocantins-Araguaia Basin. In Tocantins and Araguaia River's, the accidents occur especially during the dry season (June to September), when river waters decrease and stingrays approach the banks, and the bathers (Santos et al., 2014). In this region occur at least eight Potamotrygonidae´s species including *Potamotrygon rex* (Guedes, 2021). To better understand of those animals, this study aimed to describe the histology and histochemistry of the integument covering the back and sting of the freshwater stingray *Potamotrygon rex*.

2. Material and Methods

2.1. Animals

Samples were collected from the integument of the back and stinger of six individuals of the freshwater stingray *Potamotrygon rex,* in the Middle Upper Tocantins River, municipality of Porto Nacional, Tocantins - Brazil (S10°41'5. 23068"; W48º25'11. 85024"). For histological analysis, five distinct regions of the animal's back were extracted: median tail, base of the tail, cephalic region, left lateral fin, right lateral fin (Figure 1A). The stinger was separated into three parts for analysis: apex, middle and base (Figure 1B). The cross-sections of the stinger were analyzed in three distinct regions: dorsal region - DR, dorsolateral region – DLR, ventrolateral region - VLR (Figure 1C). This study was conducted with authorization from SISBIO (process nº 71164-1), is registered in SisGen (process nº AE07024) and was authorized by the Ethics Committee on the Use of Animals (CEUA/UFT; process nº 23.101.006.451/2019-36).

2.2. Histology

For examination under a light microscope, samples from the animal's back, properly identified, were immediately immersed in 10% formalin in 0,1M PBS buffer (pH 7.2 – 7.4), where they remained for 24 hours. Then, they were submitted to decalcification, using 7% ethylenediamine tetraacetic acid (EDTA; pH 7.0 – 7.2), with periodic changes of the decalcifier. The stingers, remained in a 7% aqueous solution of nitric acid for approximately 15 days, changing the medium daily. Subsequently, they were dehydrated in ethyl alcohol, using increasing concentrations (70% to 100%), and embedded in paraffin. The fragments of the integument of the back and stinger were embedded in

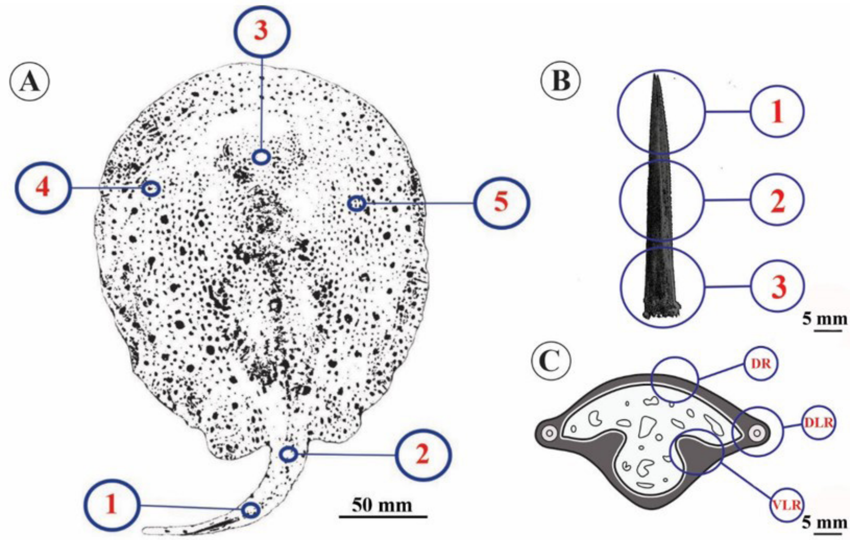

Figure 1. Collection sites for the tissues of the back and stinger of the *Potamotrygon rex* stingray, numbered according to their position. (A) Back of the stingray: 1- median tail; 2- base of the tail; 3- cephalic region; 4- left lateral fin; 5- right lateral fin. (B) Stinger: 1- apex; 2-middle and 3- base. (C) Scheme showing transversal section of the stinger regions that were analyzed under light microscopy. DR- Dorsal Region, DLR- Dorsolateral Region, VLR- Ventrolateral Region.

Leucar's molds, and oriented so that cross-sections were obtained. To describe the general morphology of the integument and stinger, the sections (5 µm thick), were stained with hematoxylin and eosin (H&E) (Suvarna et al., 2019).

2.3. Histochemistry

Histological sections were submitted to the following histochemical methods: Periodic Acid Shiff (PAS) and Alcian Blue (pH 2,5), for the identification of neutral and acidic mucopolysaccharides, respectively, and Bromophenol Blue for detection of total proteins (Suvarna et al., 2019).

The sections were observed and photographed with a DM 500 LS light microscope, equipped with a DFC420 digital camera, and an imaging program Application Suite version 3.1.0 (Leica).

3. Results

3.1. General structure

The *Potamotrygon rex* has a large and flattened back; in addition, it has dermal denticles all over the back, dark skin, with intense yellow to orange spots that extend to the tail (Figure 2A). The species has a long, thin tail with a double row of spines, where in the middle portion it has 1 to 2 stingers (Figure 2B). The body surface is made up of a continuous layer of epithelial tissue, called the integument, which is externally coated with a viscous-looking substance.

3.2. Histological and histochemical analysis of the back and stinger

The different regions of the back studied (Figure 1A) did not show relevant morphological changes, likewise, the different regions of the stinger (Figures 1B and 1C). Therefore, the histological study from the different regions of the stingray's back and stinger will be described generally. Some peculiar aspects will be mentioned.

The back and stinger integument epithelium of *P. rex* is constituted by non-keratinized stratified pavement epithelial tissue with distinct epithelial cell layers. According to the morphology of the cells present in the integument of the back and the stinger, three distinct layers were identified: superficial, intermediate and basal (Figure 3)

The layer superficial of the back is formed by 2 to 3 layers of flat and juxtaposed cells. The middle layer is formed by epithelial cells spaced apart from each other (Figures 3A, 3C). Among these cells, it is possible to notice several mucous cells, which are located close to the superficial layer. They are large and rounded cells, whose cytoplasm is filled with a large amount of acidophilic substance where the nucleus is often not observed, as it is compressed against the cell membrane (Figures 3A, 3C). Eosinophilic, homogeneous-looking areas were noted in the middle region usually below the mucus cells (Figure 3C).

Some chromatophores were observed between the epithelial cells, but they are seen in greater quantity in the connective tissue adjacent to the epithelium (Figures 3C, 3E). Granulosa cells were observed close to the basement membrane. Its nucleus has loose chromatin and is located on the periphery of the cell. The cytoplasm has eosinophilic granules located in the most peripheral region, forming a true belt (Figure 3E). The epithelial cells of the basal layer (germ layer) are cubic, with a rounded nucleus and basophilic cytoplasm and are supported on the basement membrane (Figures 3C and 3E, Table 1).

Although all the studied stingray dorsal regions presented the same morphological structure, its general appearance varied according to the observed region (Figures 4A-E). Thus, in the regions of the base of the tail (Figure 4B) and in the cephalic region (Figure 4C), the mucous cells formed several layers, increasing the

Figure 2. Adult female specimen of the stingray *Potamotrygon rex*. (A) Dorsal view of the stingray. (B) Tail of the stingray with two stingers attached to the middle portion of the tail, in the dorsal region, where the outermost stinger (*) is larger than the innermost one.

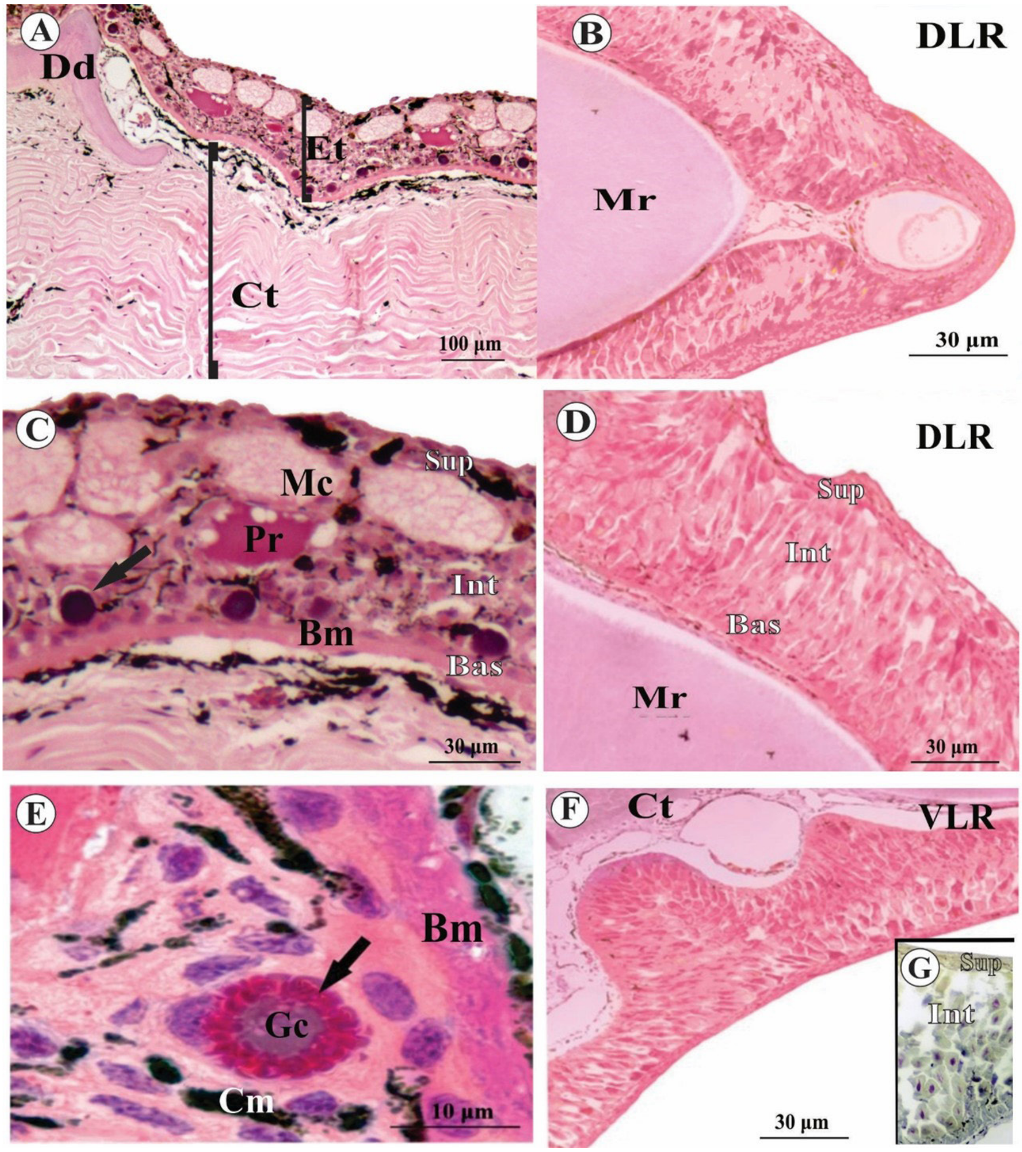

Figure 3. Photomicrograph of the back and stinger of the *Potamotrygon rex*'s epidermis. (A) General view of the back. (C) Detail of the epithelial tissue of the back showing the layers: Sup- superficial; Int- intermediary; Bas- basal. (E) Detail of the basal layer showing the granulosa cell (arrow). (B. D and F) Different regions of the stinger and the different layers of the epithelium: Sup- superficial, Int- intermediate, Bas- basal. (G) Cross section of the stinger showing the superficial (Sup) and intermediate (Int) layers, where it is possible to notice the epithelial cells of the intermediate layer. Et- epithelial tissue, Ct- connective tissue; Dd- dermal denticle; Mc- mucous cell; Gc – granulosa cell; Pr- protein reserve; Cm- chromatophores; DLR - dorsolateral region; VLR- ventrolateral region; Mr- mineralized region. (A-F) H&E stain. (G) PAS stain.

integument thickness. Protein reserves were observed in the entire integument of the stingray's back, but more concentrated in the lateral fins (Table 1).

The stinger consists of the three types of tissue: epithelial tissue, connective tissue itself, and cartilaginous tissue (Figures 3B, 3D, 3F). In epithelial tissue, the surface layer is formed by non-keratinized stratified pavement epithelial tissue. The intermediate layer has spaced epithelial cells. In the basal layer, the cubic epithelial cells are young and in direct contact with the basal membrane. Among the different layers of epithelial cells, chromatophores were observed, but mucous cells and granulosis cells were not found (Table 1; Figures 3B, 3D, 3F). The technique used for decalcification of the stinger showed a negative coloration of the nucleus in H&E, however, it was possible to observe it in PAS (Figure 3G).

The back epithelium of *P. rex* presented the same results for the histochemical reactions, for all analyzed regions (Table 2). Mucous cells were positive for PAS, as was the basal membrane (Figure 5A). In the intermediate layer the epithelial cells were mildly positive for PAS (Figure 5B) and Alcian Blue (Figure 5C). The reaction with Bromophenol

Table 1. Number of layers and cell types present in the back and stinger of the freshwater stingray *Potamotrygon rex*.

Regions	Cells Types					Reserves of Proteins
	Mucus Cell	Granulosis Cell	Epithelial Cell	Chromatophores	Specialized cells in protein synthesis	
1-Median tail	+	+	+++	+++	-	+
2- Base of the tail	++	+	+++	+++	-	+
3- Cephalic region	+++	+	+++	+++	-	++
4- Left lateral fin	+	+++	+++	+++	-	+++
5- Right lateral fin	+	+++	+++	+++	-	+++
1- Stinger apex	-	-	+++	+	++	+
2- Stinger middle	-	-	+++	+	+++	+
3- Stinger base	-	-	+++	-	++	+

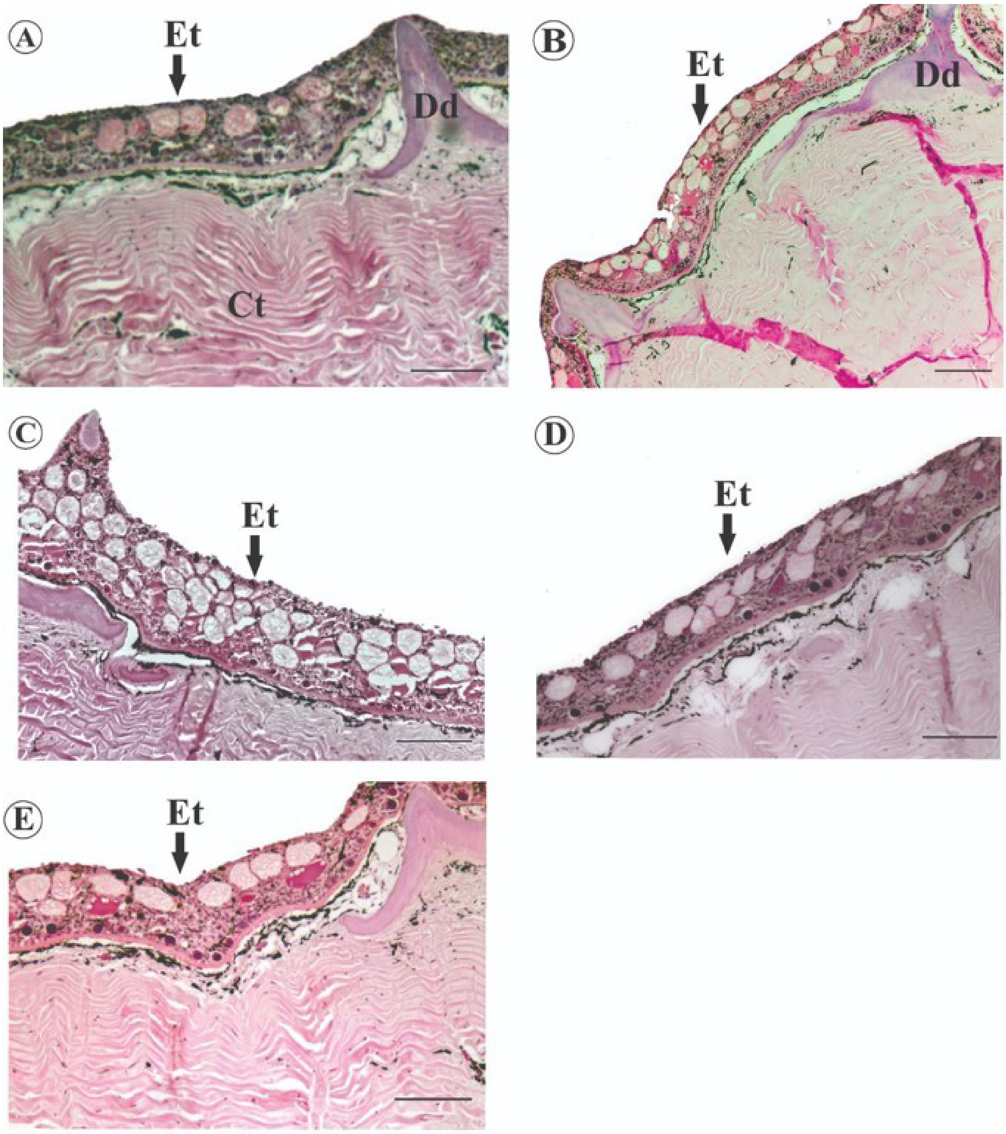

Figure 4. Micrographs demonstrating cell morphology along the back of the *Potamotrygon rex* stingray. Et- epithelial tissue, Ct- connective tissue. H&E stain. Bar = 100µm.

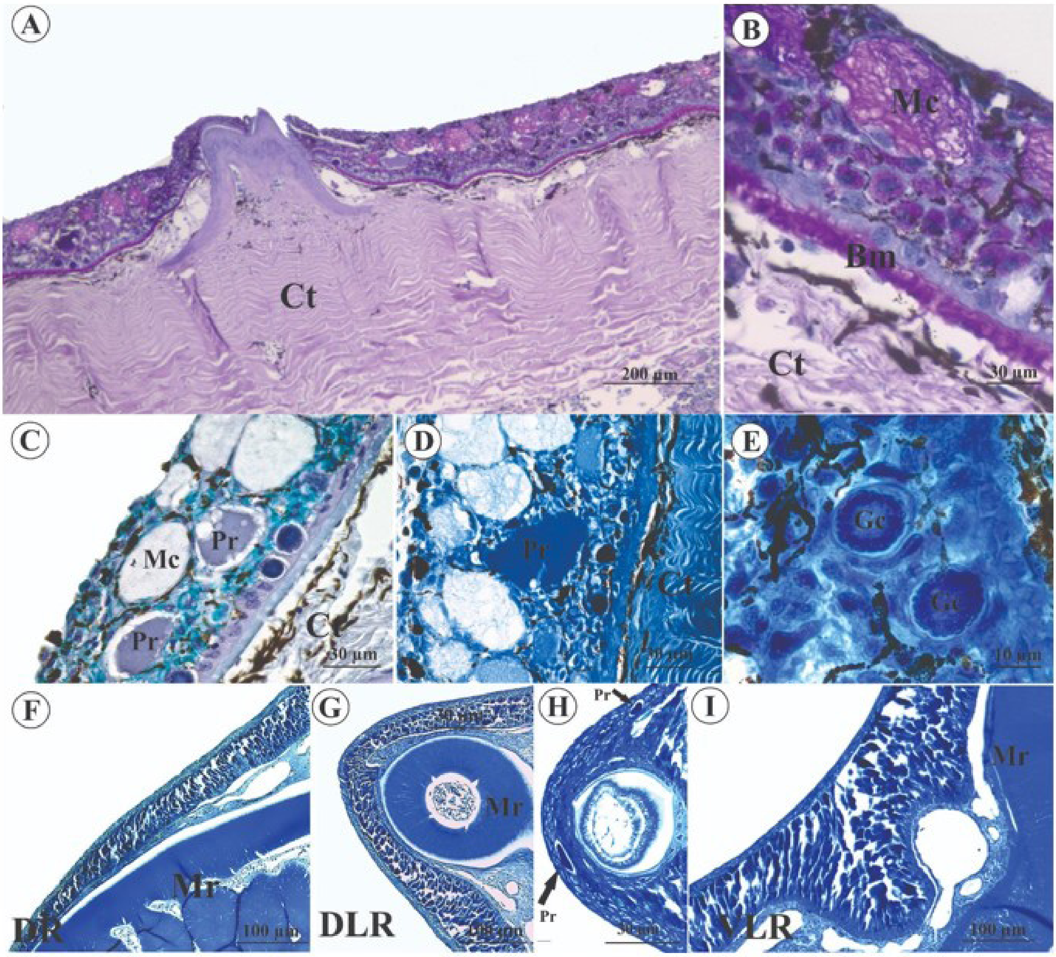

Figure 5. Histochemistry of the *Potamotrygon rex* stingray: back (A: B: C: D; E) and stinger (F; G; H, I). (A) Micrograph showing mucous cells positive by the PAS histochemical method. (B) Image magnification showing that epithelial cells also presented positive cytoplasm to PAS, as well as the basal membrane (Bm). (C) Alcian Blue positive epithelial cells. (D) Epithelial cells positive to Bromophenol Blue, detail (Pr) Protein reserve strongly positive for Bromophenol Blue. (E) granulosa cells positive to Bromophenol Blue. (F, G, H, I) Stinger regions with all Bromophenol Blue positive cells. Ct- connective tissue; Bm- basal membrane; Mc – mucous cell; Pr- protein reserve (arrows); Mr- mineralized region; Gc – granulosa cell; Cm- chromatophores; DLR - dorsolateral region; VLR- ventrolateral region.

Table 2. Main results found after H&E and histochemistry of the skin of the back and stinger of the freshwater stingray *Potamotrygon rex*.

Sample	Methods	Techniques	Main Results
Back	Histology	Hematoxylin and Eosin (H&E)	Epithelium organized in three layers: basal, intermediate and superficial Non-keratinized stratified pavement epithelial tissue
	Histochemistry	1 - Bromophenol Blue	1-Protein reserves
		2 - Alcian Blue	2- Presence of acidic mucopolysaccharides
		3 – PAS	3- Presence of neutral mucopolysaccharides
Stinger	Histology	Hematoxylin and Eosin	Epithelium organized in three layers: basal, intermediate, and superficial Non-keratinized stratified pavement epithelial tissue
	Histochemistry	1 - Bromophenol Blue	1- Presence of cells with protein-filled vesicles in the middle layer (protein cells)
		2 - Alcian Blue	2 - Absence of acidic mucopolysaccharides
		3 – PAS	3 - Absence of neutral mucopolysaccharides

Blue was strongly positive on the three layers of epithelial tissue cells, in epithelial cells of all layers, and in granules that form granulosis cells (Figure 5D, 5E).

For the histochemical reactions performed on the stinger, only Bromophenol Blue stained of the epithelial tissue, being more strongly positive in the epithelial cells that formed the intermediate layer, specialized cells in protein synthesis (Table 1 and 2; Figures 5F-I).

4. Discussion

The skin covering the back of *Potamotrygon rex* presents structures similar to the elasmobranch group, in which it is densely covered by placoid scales. Stingrays have a dorsoventrally flattened body, with eyes and spiracles located in the dorsal region (Halstead, 1988; Mceachran and Aschliman, 2004). According to Carvalho (2016), the freshwater stingray *P. rex* has unique characters, such as blackish to dark brown dorsal color, with intense yellow to orange spots, forming distinct concentric groups on the dorsal disc and tail, separated by a slender, reticulated pattern with a dark background color.

Halstead (1967) described the epithelium of marine and freshwater stingrays, and mentioned that the stinger was covered by a thin gray tissue connected with the integumentary sheath in the dermis. For Halstead (1967) the epidermis is burst under pressure and remains inside the victim's wound, causing additional trauma to the mechanical effect of the penetration of the stinger.

The integument that lines the *P. rex* stinger is constituted by three distinct layers of epithelial cells: basal, intermediate, and superficial layers, suggesting that these layers have distinct functions. The basal layer has a cell replacement function. The middle layer has epithelial cells that are probably related to the production of toxins or part of it. These are strongly positive cells for the Bromophenol Blue reaction, indicating protein synthesis activity and the presence of disulfides. The surface layer is related to the protection of the structure.

Secretory ducts were not observed in *P. rex* stinger lining cells, like what was found in other studies. Halstead (1970) named the stinger's integument cells, with large amounts of protein, as cells specialized in the production of toxins, as they participated in the constitution of the poisonous content, and classified them as holocrine cells, where the accumulation of secretion in the cytoplasm breaks down the plasma membrane leaking the cytoplasmic content to the outside of the cell. This result was contested by Smith et al. (1974), who reported not having observed evidence of holocrine secretion for these cells. Smith et al. (1974, 1981), using transmission electron microscopy, found in cells specialized in the production of *Dasyatis sabina* Lesueur, 1824, toxins a peculiar type of microtubule, with a smaller diameter than normal, and suggested that they would be involved in the secretion of protein polymers contained in the venom, therefore, it was kept isolated from the rest of the cytoplasm. In a later study, Pedroso et al. (2007) suggested that these microtubules could be used to identify this cell type. Cells specialized in the production of toxins synthesize proteins especially for internal use, the presence of electron-dense cytoplasm, full of polysomes, and the absence of secretory vacuoles were the evidence found by Pedroso et al. (2007).

The freshwater stingray *P. rex* also presented a dorsal epithelium constituted by non-keratinized stratified pavement epithelial tissue, with distinct epithelial cell layers: basal layer, intermediate layer, and superficial layer. The epithelial cells of the basal layer (germ layer) are cubic, with a round nucleus and basophilic cytoplasm, and are supported by the basal membrane. When compared to sharks, the dorsal integument of stingrays presents an irregular distribution of cells with a compact and homogeneous characteristic (Meyer and Seegers, 2012).

The superficial layer of the integument of *P. rex*, forms 2 to 3 layers of flat and juxtaposed cells. For Seegers and Meyer (2009), cells in the surface layer of the integument of stingrays have a cytoplasm with many vesicles of different densities and closely accumulated, visible only in transmission electron microscopy.

In the intermediary layer of the *P. rex* back tissue is formed by juxtaposed epithelial cells, mucous cells (goblet) that are large, voluminous, and close to the superficial layer, chromatophores, and granulosis cells. Mucous cells found in *P. rex* are similar to goblet cells common to most groups of animals and are present on the body surface of almost all fish (Whitear, 1986; Dehghani et al, 2010; Elliott, 2011b). They usually have the nucleus and peripheral organelles (Elliott, 2000). According to Whitear (1986), fish have two types of goblet cells, mucous and serous. Mucous secretions contain glycoproteins, which generally have weakly basophilic staining or remain uncolored in histological sections, whereas serous secretions contain basic proteins and, consequently, have acidophilic staining (Blackstock and Pickering, 1980; Whitear, 1986; Dehghani et al, 2010). Mucous cells are more common in fish and, studies suggest that these differentiate from epithelial cells in the lower layers of the epidermis (Whitear, 1986; Elliott, 2000). Once differentiated, they do not undergo additional mitotic division. These cells can have many functions such as protection and regulation against microorganisms, osmoregulation, in addition to assisting in gas exchange (Whitear, 1986; Elliott, 2000, 2011a).

In general, both teleosts and elasmobranchs have an intermediate layer containing several types of cells specialized for the secretion of various substances (Whitear and Moate, 1998; Meyer and Seegers, 2012). Cells like chromatophores were found in contact with all layers of the epithelial tissue of *P. rex*. Something that has also been described for elasmobranchs (Meyer and Seegers, 2012). The most common characteristic of superficial layer cells in sharks and stingrays is the exocytotic activity; they act by transporting the content of mucous vesicles placed below the apical cells to the epidermal surface (Meyer and Seegers, 2012).

Granulosis cells were observed in the back tissue of *P. rex*. These cells are analogous to saciform cells that have a nucleus located at the base, and a granular cytoplasm that stains easily with acid dyes (Elliott, 2011b). Saciform cells have been reported in cartilaginous fish such as stingrays and chimeras, and in bony fish such as polypterids (Elliott, 2000). The functions of the secretions of these cells are unknown, they usually include several types of proteins and several enzymes, serotonin, cholesterol, and other lipids described for bony fish (Elliott, 2011a). In some species of fish, saciform cells can function analogously to the granular glands in the skin of amphibians, producing secretions that are toxic or repellent to predators (Elliott, 2011b). In other fish, saciform cells can produce regulatory peptides or intraspecific alarm pheromones (Elliott, 2011b).

The epithelial cells presented in their cytoplasm the coexistence of acidic and neutral mucopolysaccharides, evidenced by positive PAS and Alcian Blue histochemical methods. The analysis of the mucus cells revealed the presence of neutral glycoconjugates and mucopolysaccharides, positive by the PAS histochemical method. We found large reserves of proteins, due to their reactivity to Bromophenol Blue, these were dispersed in the stinger and back of the stingray. As the nuclei were not visualized, there is no evidence that they are areas of protein reserve within a cell. These structures are analogous to the flask-shaped cells found in the stingers of *P. falkeri* and *P. orbignyi* stingrays, in the study by Pedroso et al. (2007).

In this study, an increase in mucous cells was observed in the integument of the back, in the regions of the base of the tail, and the head, making them thicker than in the other regions. Probably, the greatest amount of mucus is produced in the center of the animal's back, and it spreads through the body, helping in the regulation and protection process. However, mucus production in most elasmobranchs is related to environmental disturbances. The simple fact of handling a stingray provides an increase in the number of mucus cells. In general terms, these cells present very fast environmental responses to external stimuli, and everything can vary according to water quality, solar radiation, sex, among other factors (Elliott, 2011a).

The cells secretions present in the fish epithelial tissue form a layer called the cuticle on the skin surface, where they are mixed and modified (Elliott, 2011a). Potamotrygonids are benthic animals and almost always live buried under the sand (Garrone-Neto and Uieda, 2012), which allows the accumulation of decomposing organic sediments. Therefore, the stingray skin secretes substances that participate in the osmotic and ionic regulation, as well as protection against external pathogens, also helping in the locomotion and reproduction of these animals (Shephard, 1994; Zhao et al., 2008).

The histochemical study of this work showed that all cells of the intermediate and superficial layers, present in the epithelial tissue of the *P. rex* back, participate in the production of the cuticle, confirmed by the coexistence of acidic and neutral mucins, as well as total proteins and disulfides in the epidermal cells, mucopolysaccharides, and glycoconjugates in mucous cells, in addition to large protein reserves dispersed throughout the integument.

The cuticle produced by the lining tissue cells of stingrays has great molecular diversity such as amino acids, carbohydrates, glycopeptides, glycolipids, among others. The discovery of the pentraxin lectin in the epidermal mucus of the marine stingray *Okamejei kenojei* Müller & Henle, 1841 (Tsutsui et al., 2009), demonstrates the role of the epidermis in the skin's basic defense against pathogenic diseases caused by bacterial invasion. Furthermore, new peptides such as Orpotrin and Porfflan were discovered in the mucus of freshwater stingray *Potamotrygon gr. orbignyi* Castelnau, 1895 (Conceição et al., 2006, 2009). As well as, a new antimicrobial protein present in the mucus of the stingray *Potamotrygon cf. henlei* (Conceição et al., 2012), species described as *P. rex* by Carvalho (2016). Little is known about the mechanisms of action conveyed to the production, secretion, and dispersion of these substances, as well as their physiological function for the animal. However, it is known that both the mucus present on the back and the stinger of this species have toxic activity (Monteiro-dos-Santos et al., 2011). In addition, the serum produced from *Paratrygon aireba* mucus can neutralize the main symptoms of stingray poisoning (Thomazi et al., 2020).

Cuticle production is part of the defense of these animals against external agents, given their habitat and benthic behavior. The epidermal cells on the back of stingrays produce molecules that participate in the defense and regulation process in the animal's body; however, some may be the same or like those produced in the stinger.

5. Conclusion

Therefore, the tissues that line the back and the stinger of *Potamotrygon rex* show similarities and differences. Both are formed by non-keratinized stratified pavement epithelial tissue with three cell layers (basal, intermediate, and superficial). However, the epithelial tissue covering the back consists of a more diverse set of cells (epithelial cells, mucus cells, granulosis cells, chromatophores) compared to the stinger tissue (epithelial cells, specialized cells and chromatophores).

Thus, the integument covering the back is organized to produce the cuticle, which wraps around the animal's body. While the rigid structure of the stinger, capable of piercing/cutting tissue, is lined by tissue organized to produce proteins, especially those cells located in the epithelium intermediate layer.

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